

ROLE OF TTF-1, CK20, AND CK7 IMMUNOHISTOCHEMISTRY FOR DIAGNOSIS OF PRIMARY AND SECONDARY LUNG ADENOCARCINOMA

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Thyroid transcription factor-1 (TTF-1), and cytokeratin 7 (CK7) and cytokeratin 20 (CK20) have recently been reported to be useful to distinguish between primary and metastatic lung adenocarcinoma. The aim of this study was to determine the usefulness of the staining patterns of pulmonary adenocarcinoma with antibodies to TTF-1, CK7, and CK20 in differentiating primary from metastatic pulmonary adenocarcinoma. Of the 66 lung adenocarcinoma specimens that were enrolled in our study, there were 40 primary lung adenocarcinomas, 12 metastatic adenocarcinomas from breast, 13 metastatic adenocarcinomas from colon, and 1 metastatic adenocarcinoma from stomach. The expression of TTF-1, CK7, and CK20 was assessed by immunohistochemistry. We found that 73% of primary lung adenocarcinomas expressed TTF-1, whereas all nonpulmonary adenocarcinomas lacked TTF-1 staining. CK7 expression was significantly more frequent in adenocarcinomas of pulmonary and breast origin than gastrointestinal (GI) origin ($p < 0.001$). In contrast, CK20 expression was significantly more prevalent in adenocarcinoma that originated in the GI tract than that of pulmonary or breast origin ($p < 0.001$). A combination of TTF-1⁺CK7⁺CK20⁻ was highly significantly associated with primary adenocarcinoma of lung (vs GI tract, $p < 0.001$; vs breast, $p < 0.001$). A combination of TTF-1⁻CK7⁻CK20⁺ was highly significantly associated with adenocarcinoma of GI origin (vs lung, $p < 0.001$; vs breast, $p < 0.001$). Our study has confirmed that expression of CK7, CK20, and TTF-1 is a useful immunohistochemical marker for diagnosis of lung tumors and for differential diagnosis of primary pulmonary adenocarcinomas from extrapulmonary adenocarcinomas metastatic to the lung. Application of this panel of antibodies might be expected to increase the accuracy of diagnosis.

Key Words: TTF-1, CK7, CK20, immunohistochemistry, lung adenocarcinoma
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A common diagnostic problem remains in differentiating by routine histology primary pulmonary adenocarcinoma from adenocarcinoma metastatic to the lung (eg, originating from breast, gastric, colon, thyroid, pancreas or renal cancers) [1,2]. Pathologists are often asked to identify the primary site. It has been reported that immunohistochemistry is a

useful method for ascertaining the site of origin in such cases. A review of recent studies reveals that the immunohistochemical markers surfactant protein-B, PE-10, thyroid transcription factor-1 (TTF-1), cytokeratin 20 (CK20), and cytokeratin 7 (CK7) have efficacy for discriminating between primary and metastatic pulmonary adenocarcinoma [1-5]. In clinical situations, because the most prevalent sites of adenocarcinoma are in the lungs, colon, and breast, a combined analysis of TTF-1, CK7, and CK20 expression may have high sensitivity and specificity for the separation of primary lung adenocarcinomas from metastatic adenocarcinomas of colonic or breast origin [1-4].

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TTF-1 is a member of the NKX2 family of homeodomain transcription factors [6]. It is a 38-kDa protein, located primarily in the nuclei of type II pneumocytes and Clara's cells in the lung, thyroid tissues, and the diencephalons of the brain [7,8]. In the lung, it binds to and helps regulate Clara's cell secretory and surfactant A, B, and C protein gene expression [9–11]. In addition, it has also been observed in 75–80% of pulmonary adenocarcinomas [1].

Although both CK7 and CK20 are intermediate filaments, they are located in different epithelial cells. CK7, a 54-kDa basic protein, is typically located in epithelia from lung and breast; CK20, a 46-kDa acidic cytokeratin protein, is common in epithelia from the intestinal tract [2].

Thus, in this present study, we studied the staining patterns of pulmonary adenocarcinoma with antibodies to TTF-1, CK7, and CK20, so as to confirm the usefulness of this panel in differentiating primary from metastatic pulmonary adenocarcinoma.

MATERIALS AND METHODS

Tissue specimens were obtained from 66 cases of lung adenocarcinoma diagnosed at the Department of Pathology, Kaohsiung Medical University Chung-Ho Memorial Hospital, between 1998 and 2004. All tumors were surgical resection specimens. There were 40 primary pulmonary adenocarcinomas, 12 metastatic adenocarcinomas from breast, 13 metastatic adenocarcinomas from colon, and 1 metastatic adenocarcinoma from stomach.

Formalin-fixed, paraffin-embedded tissue specimens were investigated. For immunostaining, deparaffinized and rehydrated sections were heated in an oven at 121°C for 30 minutes in citrate buffer to retrieve antigenic activity and then cooled at room temperature. Endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxide in methanol for 20 minutes. After nonspecific reactions had been blocked with 10% normal bovine serum, the sections were incubated with monoclonal antibodies specific to TTF-1 (1:50; M3575; DAKO), to CK7 (1:50; M7018; DAKO), and to CK20 (1:50; M7019; DAKO) for 40 minutes. The sections were then incubated with biotinylated goat anti-mouse immunoglobulin (Ig) for 20 minutes and then with streptavidin–peroxidase complex for 20 minutes. Careful rinses were performed with several changes of phosphate-buffered saline between each stage of the procedure. Finally, the color was developed with diaminobenzidine followed by hematoxylin counterstaining and mounting. Negative controls were performed by

replacing the primary antibody with nonimmune mouse IgG.

The immunostained specimens were evaluated by two observers (C-Y.C. and Y-C.S.) without previous knowledge of their clinicopathologic features. Tumors were considered negative if staining was found in fewer than 10% of neoplastic cells and positive if present in more than 10%. The results of immunostaining with CK7 and CK20 were based on cytoplasmic staining of neoplastic cells, whereas nuclear staining was used for TTF-1 (Figure 1A–D).

The χ^2 test or the Fisher exact test was used to analyze each two-dimensional table of discrete data. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

The patients ranged in age from 22 to 82 years; 26 were men and 40 were women. TTF-1, CK7, and CK20 positivity was shown in 73% (29/40), 75% (30/40), and 0% (0/40) of primary pulmonary adenocarcinomas, respectively. TTF-1, CK7, and CK20 positivity was shown in 0% (0/12), 50% (6/12), and 0% (0/12) of secondary adenocarcinomas that originated in the breast, respectively. TTF-1, CK7, and CK20 positivity was shown in 0% (0/12), 7% (1/14), and 86% (12/14) of secondary adenocarcinomas that originated in the colon, respectively. All nonpulmonary adenocarcinomas lacked TTF-1 staining. CK7 expression was significantly more frequent in adenocarcinoma of pulmonary and breast origin than that of GI tract origin (*p* < 0.001, Table 1). In contrast, CK20 expression was significantly more prevalent in adenocarcinoma of GI tract origin than that of pulmonary and breast origin (*p* < 0.001, Table 1).

Tumors from the lung, breast, and GI tract were also compared by evaluating a combination panel of all three immunostains (TTF-1, CK7, CK20, Table 2). A combination of TTF-1 positivity, CK7 positivity, and CK20 negativity was highly significantly associated with primary pulmonary adenocarcinoma compared with any of the other tumor types (*p* < 0.001). The sensitivity and specificity of this combination of immunomarkers for a primary lung adenocarcinoma were 60% and 100%, respectively. A combination of TTF-1 negativity, CK7 negativity, and CK20 positivity was highly significantly associated with adenocarcinoma of GI origin compared with either of the other tumor types (vs lung, *p* < 0.001; vs breast, *p* < 0.001). The sensitivity and specificity of this combination of immunomarkers for a secondary adenocarcinoma from the GI tract were 79% and 100%, respectively.

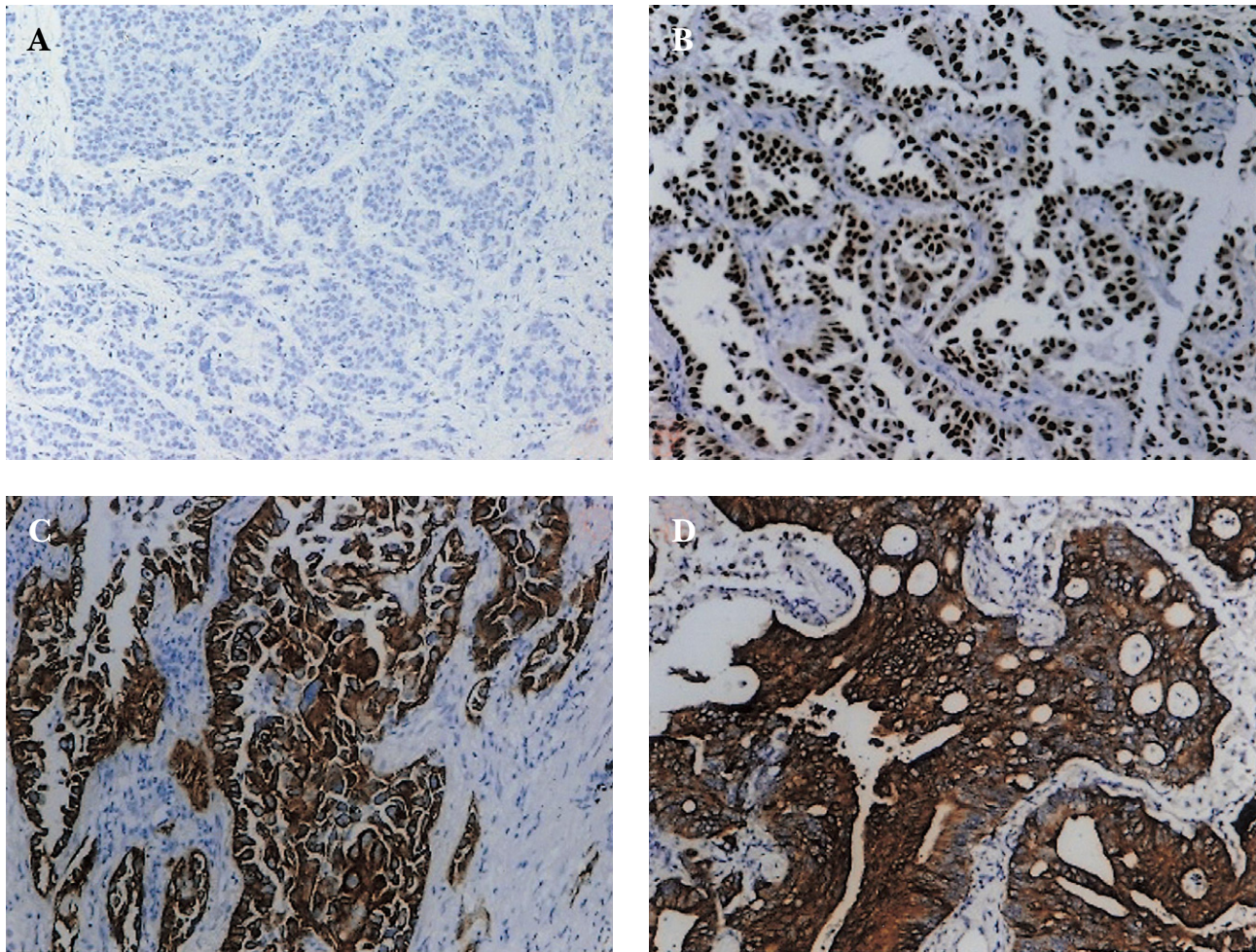


Figure 1. (A) Negative control. (B) Primary lung adenocarcinoma showing strong staining for TTF-1. (C) Breast origin lung adenocarcinoma showing strong staining for CK7. (D) Colon origin lung adenocarcinoma showing strong staining for CK20. (A–D, original magnification $\times 200$).

Table 1. Summary of TTF-1, CK7, and CK20 expression in primary and secondary adenocarcinoma of lung

Immunomarker	Lung origin (n = 40)	Breast origin (n = 12)	GI tract origin (n = 14)
TTF-1			
Positive	29 (73%)*	0 (0%)	0 (0%)
Negative	11 (27%)	12 (100%)	14 (100%)
CK7			
Positive	30 (75%) [†]	6 (50%) [‡]	1 (7%)
Negative	10 (25%)	6 (50%)	13 (93%)
CK20			
Positive	0 (0%)	0 (0%)	12 (86%) [§]
Negative	40 (100%)	12 (100%)	2 (14%)

TTF-1 = thyroid transcription factor-1; CK7 = cytokeratin 7; CK20 = cytokeratin 20.

*Lung (73%) vs breast (0%) or lung (73%) vs GI tract (0%), $p < 0.001$.

[†]Lung (75%) vs GI tract (7%), $p < 0.001$.

[‡]Breast (50%) vs GI tract (7%), $p < 0.001$.

[§]GI tract (86%) vs breast (0%) or GI tract (86%) vs lung (0%), $p < 0.001$.

Table 2. Summary of TTF-1/CK7/CK20 immunomarker panels in primary and secondary adenocarcinoma of lung

Panel of immunomarker	Lung origin* (n = 40)	Breast origin (n = 12)	GI tract origin (n = 14)
1. TTF-1 ⁺ CK7 ⁺ CK20 ⁺	0	0	0
2. TTF-1 ⁺ CK7 ⁺ CK20 ⁻	24 (60%) [†]	0	0
3. TTF-1 ⁺ CK7 ⁻ CK20 ⁺	0	0	0
4. TTF-1 ⁺ CK7 ⁻ CK20 ⁻	5 (13%)	0	0
5. TTF-1 ⁻ CK7 ⁺ CK20 ⁺	0	0	1 (7%)
6. TTF-1 ⁻ CK7 ⁺ CK20 ⁻	6 (15%)	6 (50%)	0
7. TTF-1 ⁻ CK7 ⁻ CK20 ⁺	0 [‡]	0 [§]	11 (79%)
8. TTF-1 ⁻ /CK7 ⁻ /CK20 ⁻	5 (13%)	6 (50%)	2 (14%)

TTF-1 = thyroid transcription factor-1; CK7 = cytokeratin 7; CK20 = cytokeratin 20.

*Sensitivity/specificity for lung origin adenocarcinomas panels: 1, 0%/100%; 2, 60%/100%; 3, 0%/100%; 4, 5%/100%; 5, 0%/96%; 6, 15%/77%; 7, 0%/58%; 8, 3%/69%.

[†]Lung vs breast or GI tract, $p < 0.001$; sensitivity 50%, specificity 100%.

[‡]GI tract vs lung, $p < 0.001$.

[§]GI tract vs breast, $p < 0.001$.

DISCUSSION

It is important to distinguish between primary pulmonary adenocarcinoma and metastatic pulmonary adenocarcinoma, because the treatment and prognosis differ considerably for patients with these malignancies. In the study presented here, we compared three antibodies—TTF-1, CK7, and CK20—as markers of primary and metastatic lung adenocarcinoma. Our results determined the sensitivity and specificity of the antibodies as markers of primary and metastatic epithelial lung tumors. In addition, the use of a combination panel of these three immunostains provides valuable information for the differential diagnosis of lung adenocarcinoma.

According to many previous studies, TTF-1 is a tissue-specific gene expression marker that has been reported to play a crucial role in the molecular pathogenesis of primary adenocarcinoma of the lungs [12]. In addition, some studies found that the extent of differentiation and histologic features correlated with the intensity of TTF-1 staining; for example, poorly differentiated and solid tumors producing mucin were less likely to stain [12–14]. Recently, those cases with positive TTF-1 expression have also been found to be associated with better patient survival than those with negative TTF-1 expression [15]. Our study found that TTF-1 has not only high sensitivity but also high specificity for primary adenocarcinoma of the lungs. Thus, for clinical practice, TTF-1 could serve as a reliable marker for diagnosis of primary adenocarcinoma of the lungs.

CK20 has demonstrated activity specific for the normal intestinal epithelium, the gastric foveolar epithelium, the umbrella cells of the urothelium, and epidermal Merkel cells [16]. Thus the antibody labels the majority of adenocarcinomas of the colon, mucinous ovarian tumors, and transitional and Merkel cell carcinomas, and it is often positive in adenocarcinoma of the stomach, bile duct, gallbladder, and pancreas [17]. Adenocarcinomas of the breast, lung, and ovary are essentially negative [17]. The most frequent CK20⁺ carcinoma type is adenocarcinoma of the colon and rectum; however, as the current study shows, a considerable proportion of colorectal carcinomas do not react with CK20. Although CK20 positivity (particularly when it is coupled with CK7 negativity) strongly indicates a colorectal origin, CK20 negativity (with or without CK7 negativity) does not necessarily rule out this possibility [18–20].

CK7 is known to label several types of normal and neoplastic glandular epithelia; carcinomas from the GI tract and prostate generally are negative, whereas breast, lung, and subtypes of adenocarcinomas of the ovary generally are positive [21]. In this study, we found that CK7 staining was equivocal for adenocarcinoma of breast origin, and it also could be rarely expressed by secondary adenocarcinoma of the GI tract (7%).

Since the early 1990s, the clinical utility of monoclonal antibodies directed against CK20 paired with anti-CK7 antibodies in the differential diagnosis of primary lung cancers and secondary lung cancers has been reported [22].

Adenocarcinoma from the colon is usually CK20⁺ and CK7⁻, whereas adenocarcinoma from the lung is usually CK20⁻ and CK7⁺ [3].

There seems to be a strong indication in the literature that the use of an antibody panel combining CK7, CK20, and TTF-1 in differentiating between primary and metastatic adenocarcinomas of the lung is reliable [2]. In our study, we noted that, when TTF-1 was used in conjunction with CK7 and CK20, it was useful in discriminating between primary and metastatic adenocarcinomas of the lung. The combination of the CK7⁺CK20⁻ immunophenotype, along with TTF-1 immunoreactivity, was highly specific for primary pulmonary adenocarcinoma (specificity 100%).

CONCLUSIONS

Our study has confirmed that CK7, CK20, and TTF-1 expression are useful as immunohistochemical markers for the diagnosis of lung tumors and for the differential diagnosis of primary pulmonary adenocarcinomas from extrapulmonary adenocarcinomas metastatic to the lung. Application of this panel of antibodies might be expected to increase the accuracy of diagnosis.

REFERENCES

1. Moldvay J, Jackel M, Bogos K, et al. The role of TTF-1 in differentiating primary and metastatic lung adenocarcinomas. *Pathol Oncol Res* 2004;10:85–8.
2. Cai YC, Banner B, Glickman J, Odze RD. Cytokeratin 7 and 20 and thyroid transcription factor 1 can help distinguish pulmonary from gastrointestinal carcinoid and pancreatic endocrine tumors. *Hum Pathol* 2001;32:1087–93.
3. Chieng DC, Cangiarella JF, Zakowski MF, et al. Use of thyroid transcription factor 1, PE-10, and cytokeratins 7 and 20 in discriminating between primary lung carcinomas and metastatic lesions in fine-needle aspiration biopsy specimens. *Cancer* 2001;93:330–6.
4. Jang KY, Kang MJ, Lee DG, Chung MJ. Utility of thyroid transcription factor-1 and cytokeratin 7 and 20 immunostaining in the identification of origin in malignant effusions. *Anal Quant Cytol Histol* 2001;23:400–4.
5. Zou SM, Lin DM, Lu N, et al. Use of thyroid transcription factor-1, surfactant protein-B, cytokeratin 7 and cytokeratin 20 in discrimination between primary and metastatic adenocarcinoma of lung. *Zhonghua Yi Xue Za Zhi* 2003;83:1350–2. [In Chinese]
6. Bingle CD. Thyroid transcription factor-1. *Int J Biochem Cell Biol* 1997;29:1471–3.
7. Khoor A, Whitsett JA, Stahlman MT, et al. Utility of surfactant protein B precursor and thyroid transcription factor 1 in differentiating adenocarcinoma of the lung from malignant mesothelioma. *Hum Pathol* 1999;30:695–700.
8. Lazzaro D, Price M, de Felice M, Di Lauro R. The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. *Development* 1991;113:1093–104.
9. Bohinski RJ, Di Lauro R, Whitsett JA. The lung-specific surfactant protein B gene promoter is a target for thyroid transcription factor 1 and hepatocyte nuclear factor 3, indicating common factors for organ-specific gene expression along the foregut axis. *Mol Cell Biol* 1994;14:5671–81.
10. Bruno MD, Bohinski RJ, Huelsman KM, et al. Lung cell-specific expression of the murine surfactant protein A (SP-A) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. *J Biol Chem* 1995;270:6531–6.
11. Zhang L, Whitsett JA, Stripp BR. Regulation of Clara cell secretory protein gene transcription by thyroid transcription factor-1. *Biochim Biophys Acta* 1997;1350:359–67.
12. Jerome Marson V, Mazieres J, Groussard O, et al. Expression of TTF-1 and cytokeratins in primary and secondary epithelial lung tumours: correlation with histological type and grade. *Histopathology* 2004;45:125–34.
13. Stenhouse G, Fyfe N, King G, et al. Thyroid transcription factor 1 in pulmonary adenocarcinoma. *J Clin Pathol* 2004;57:383–7.
14. Inamura K, Satoh Y, Okumura S, et al. Pulmonary adenocarcinomas with enteric differentiation: histologic and immunohistochemical characteristics compared with metastatic colorectal cancers and usual pulmonary adenocarcinomas. *Am J Surg Pathol* 2005;29:660–5.
15. Myong NH. Thyroid transcription factor-1 (TTF-1) expression in human lung carcinomas: its prognostic implication and relationship with expressions of p53 and Ki-67 proteins. *J Korean Med Sci* 2003;18:494–500.
16. Moll R, Schiller DL, Franke WW. Identification of protein IT of the intestinal cytoskeleton as a novel type I cytokeratin with unusual properties and expression patterns. *J Cell Biol* 1990;111:567–80.
17. Moll R, Lowe A, Laufer J, Franke WW. Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. *Am J Pathol* 1992;140:427–47.
18. Ascoli V, Taccogna S, Scalzo CC, Nardi F. Utility of cytokeratin 20 in identifying the origin of metastatic carcinomas in effusions. *Diagn Cytopathol* 1995;12:303–8.
19. Loy TS, Calaluce RD. Utility of cytokeratin immunostaining in separating pulmonary adenocarcinomas from colonic adenocarcinomas. *Am J Clin Pathol* 1994;102:764–7.
20. Tot T. Adenocarcinomas metastatic to the liver: the value of cytokeratins 20 and 7 in the search for unknown primary tumors. *Cancer* 1999;85:171–7.
21. Loy TS, Calaluce RD, Keeney GL. Cytokeratin immunostaining in differentiating primary ovarian carcinoma from metastatic colonic adenocarcinoma. *Mod Pathol* 1996;9:1040–4.
22. Wauters CC, Smedts F, Gerrits LG, et al. Keratins 7 and 20 as diagnostic markers of carcinomas metastatic to the ovary. *Hum Pathol* 1995;26:852–5.

TTF-1、CK7 和 CK20 的免液組織 化學染色表現在診斷原發性 或轉移性肺腺癌的相關性研究

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TTF-1、CK7 和 CK20 最近被報導可用於臨床上辨別肺腺癌的原發器官，於是本篇研究的目的乃是根據這一個想法，想要去確認 TTF-1、CK7 和 CK20 三個因子的表現確實有利於區分肺腺癌是原發性的亦或是由其他器官如胃腸或乳房轉移而來的。選取的肺腺癌有 66 個，40 個是原發於肺臟，12 個是來自乳房轉移，13 個是來自大腸轉移，1 個是來自胃轉移，我們利用免液組織化學染色的方法來確認 TTF-1、CK7 和 CK20 的表現。73% 的原發性肺腺癌表現 TTF-1，但在所有非原發性肺腺癌則皆不表現 TTF-1；CK7 在原發性肺腺癌或來自乳房轉移的肺腺癌的表現比來自大腸轉移的肺腺癌更具有統計上的相關 ($p < 0.001$)；CK20 在來自胃腸轉移的肺腺癌的表現比在原發性肺腺癌或來自乳房轉移的肺腺癌更具有統計上的相關 ($p < 0.001$)；當呈現 TTF-1+/CK7+/CK20- 時則被認為多來自肺原發性的腺癌 (✓ 來自胃腸肺腺癌， $p < 0.001$ ；來自乳房肺腺癌， $p < 0.001$)；當呈現 TTF-1-/CK7-/CK20+ 時則被認為多來自胃腸轉移的腺癌。我們的結論是，我們認為聯合 TTF-1、CK7 和 CK20 三個因子的表現，確實有利於區分肺腺癌是原發性的亦或是由其他器官如胃腸或乳房轉移而來的，利用這個方法也有利於增加診斷的準確率。

關鍵詞：TTF-1，CK7，CK20，免液組織化學染色，肺腺癌

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